Video Article

Simultaneous Recordings of Cortical Local Field Potentials, Electrocardiogram, Electromyogram, and Breathing Rhythm from a Freely Moving Rat

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Abstract

Monitoring the physiological dynamics of the brain and peripheral tissues is necessary for addressing a number of questions about how the brain controls body functions and internal organ rhythms when animals are exposed to emotional challenges and changes in their living environments. In general experiments, signals from different organs, such as the brain and the heart, are recorded by independent recording systems that require multiple recording devices and different procedures for processing the data files. This study describes a new method that can simultaneously monitor electrical biosignals, including tens of local field potentials in multiple brain regions, electrocardiograms that represent the cardiac rhythm, electromyograms that represent awake/sleep-related muscle contraction, and breathing signals, in a freely moving rat. The recording configuration of this method is based on a conventional micro-drive array for cortical local field potential recordings in which tens of electrodes are accommodated, and the signals obtained from these electrodes are integrated into a single electrical board mounted on the animal's head. Here, this recording system was improved so that signals from the peripheral organs are also transferred to an electrical interface board. In a single surgery, electrodes are first separately implanted into the appropriate body parts and the target brain areas. The open ends of all of these electrodes are then soldered to individual channels of the electrical board above the animal's head so that all of the signals can be integrated into the single electrical board. Connecting this board to a recording device allows for the collection of all of the signals into a single device, which reduces experimental costs and simplifies data processing, because all data can be handled in the same data file. This technique will aid the understanding of the neurophysiological correlates of the associations between central and peripheral organs.

Video Link

The video component of this article can be found at https://www.jove.com/video/56980/

Introduction

The central nervous system controls body states in response to various environmental changes, and this control is typically represented as changes in heart rate, breathing rate, and muscle contractions. However, few studies have tested how such peripheral physiological factors are associated with cortical activity. To address this issue, a large-scale recording method for monitoring electrical biosignals from both central and peripheral tissues is necessary. In the cerebral cortex, local field potential (LFP) signals are extracellularly recorded by electrodes that are inserted into the cortical tissues[1,2,3]. To simultaneously record multiple LFP signals from the cortical regions of small mammals, such as rats and mice, a number of studies have developed various types of custom-made electrode assemblies that are termed micro-drives. A conventional micro-drive is composed of metal screws attached to the middle parts of the electrodes (which are typically tetrodes), a core body that accommodates the screws and electrodes, and an electrical interface board (EIB) that accommodates metal holes to connect the open ends of the electrodes (**Figure 1**, **Figure 2**, and **Figure 3**). This electrode assembly enables the operator to control the depth of many electrodes inserted into the brain over the course of days to weeks, and allows the conducting of long-term chronic recordings of neuronal activity as the animal is challenged with various behavioral tasks. In the peripheral organs, heartbeat signals are recorded as electrocardiograms (ECGs) by a pair of electrodes that are implanted on or around the heart area[4,5,6], and skeletal muscle signals are recorded as electromyograms (EMGs) with electrodes that are inserted into the muscle tissue[7,8,9]. The relationship between electrical signals of the olfactory bulb and breathing (BR) rhythm has been studied with single unit recordings[10,11]. In conventional recording systems, these signals from different tissues have been captured by independent recording devices, which means that an additional experimental system is required to precisely synchronize these multiple devices for simultaneous recordings of brain-body signals. This system was developed to overcome this issue. In this system, all electrical signals recorded from the peripheral organs, including ECGs, EMGs, and electrical signals from the olfactory bulb that reflect the breathing rhythm, are integrated into a single micro-drive array[1,2,3], here termed an integrative micro-drive array. This system requires only one multi-channel recording device, and is applicable to any conventional micro-drive array. The advantages of this technique are that it does not require any special devices or trigger signals to match the recording time of multiple devices, and it allows for more convenient data processing, since all of the signals are recorded as similar data types. This technique will aid the understanding of the neurophysiological correlates of the associations between central

and peripheral organs. This paper describes the procedures associated with the technique and presents representative datasets obtained from a rat.

Protocol

All of the procedures involving animal subjects were performed according to the NIH guidelines for the care and use of animals.

1. Preparation of the Integrative Micro-Drive Array

1. Create a micro-drive array for cortical LFP recordings as described elsewhere[1,2,3]. Leave at least 6 metal holes open on an electrode interface board (EIB) for use as ECG/EMG/BR channels that are connected to bioflex wires as described in 1.2.
2. Cut a bioflex wire into 6 pieces with lengths of 5.0 cm. Peel off the polytetrafluoroethylene (PTFE) coating of both ends of all the wire pieces with lengths of ~5.0 mm. Connect one end of each of the wire pieces to one of the open metallic holes (ECG/EMG/BR channels) on the EIB with a gold pin.
3. Cut an enamel wire into two 5.0-cm pieces. Solder one end of each of these wires to the ground/reference (g/r) channels on the EIB (**Figure 3**, see also previous papers[12,13]).
4. For the preparation of ECG electrodes, cut a bioflex wire into two 16-cm pieces. Peel off the PTFE coating of the ends of these wire pieces at lengths of ~5.0 mm on one end (short end) and ~15 mm on the other end (long end).
5. Form a wire ring with a diameter of 2.0 mm by bending the long end of the wire, and fixing the shape of the ring by soldering.
6. For the preparation of EMG electrodes, cut a bioflex wire into 2 pieces with lengths of 8 cm. Peel off the PTFE coating from both ends of these wire pieces with lengths of ~5.0 mm.
7. For the preparation of BR electrodes, cut a bioflex wire into 2 pieces with lengths of 6.0 cm. Peel off the enamel coating of both ends of these wire pieces with lengths of ~5.0 mm. Solder one end of each of these wire pieces to the head of a stainless- steel screw (stem diameter: 1.0 mm, stem length: 4.0 mm).
8. For the preparation of ground/reference (gr) electrodes, cut an enamel wire into 2 pieces with lengths of 6.0 cm. Peel off the enamel coating of both ends of these wire pieces with lengths of ~5.0 mm. Solder one end of each of these wire pieces to the head of a stainless- steel screw (stem diameter: 1.4 mm, stem length: 3.0 mm).
9. Gas sterilize all electrodes and stainless screws and keep these in a clean space.

2. Implantation of the ECG/EMG Electrodes

NOTE: Perform all surgical steps with aseptic technique using sterilized gloves and autoclaved instruments. For all steps involving the creation of an incision, sterilize the skin with 70% ethanol before, and cover the incision with surgical drapes.

1. Fix an anesthetized (1.0-3.0% isoflurane gas) rat on its back on a flat heat pad. Give buprenorphine as an analgesic. Place veterinary ointment on the rat's eyes to prevent dryness. Use betadine to clean the surface of the skin.
2. Make an incision of ~2.0 cm in the medial chest area. Expose the intercostal muscles by separating the chest muscles. Suture the rings of the ECG electrodes to the intercostal muscles.
3. Fix the stomach of the animal on the heat pad. Make an incision of ~1.0 cm in the dorsal neck area.
4. Insert the ECG electrodes subcutaneously through the chest incision. Slide the ends to the dorsal neck area, and pull them out from the neck incision. Suture the chest incision.
5. Insert one end of each of the EMG electrodes subcutaneously to a length of ~2.0 cm through the neck incision. Fix the EMG electrodes to the neck muscle by suturing.

3. Implantation of the Integrative Micro-Drive Array and the BR Electrodes

1. Fix the rat on a stereotaxic device. Make an incision of ~3.0 cm on the head along the midline from the point between the eyes to the neck area. Expose the skull.
2. Make two circular craniotomies with diameters of 0.7-1.0 mm above the olfactory bulb 11.0 mm anterior and 1 mm bilateral to bregma with a high-speed drill. Implant two BR electrodes in the skull until the tips of the screw stems are attached to the brain surface.
3. Make two circular craniotomies with diameters of 0.7-1.0 mm above the frontal cortex 2.7 mm anterior and 2.7 mm bilateral to bregma. Implant two g/r electrodes in the skull until the tip of the screw stem is attached to the brain surface.
4. Make six to eight holes with diameters of 1.0 mm in the area surrounding the large craniotomy. Implant anchor screws (stem diameter: 1.4 mm, stem length: 3.0 mm) in the skull.
5. Make a large circular craniotomy with a diameter of ~2.0 mm above the hippocampus 3.8 mm posterior and 2.5 mm bilateral to bregma. Place the integrative micro-drive array such that the cannula tip of the drive array is located above the large craniotomy
6. Fill the gap space between the cannula tip and the brain surface with ~100 µL of two solutions, *i.e.*, 0.5% (by mass) sodium alginate and 10% (by mass) calcium chloride.
   NOTE: This process forms a transparent gel in ~5 min, after the two solutions are mixed on the skull.
7. Cover the cannula, BR electrodes, g/r electrodes, and anchor screws with dental cement with a thickness of 0.5 cm. Be careful **NOT** to cover the open ends of BR and g/r electrodes with the cement at this step.
8. Solder the open ends of the ECG, EMG, BR and g/r electrodes to the individual wire tips that were previously connected to the EIB (see the steps 1.2 and 1.3).
9. Cover the bottom part of the integrative micro-drive array, and all electrode wires, with dental cement. Ensure that all the electrode wires are **completely covered** so that the rat cannot scratch them out after the implantation.
10. After regaining sufficient consciousness to maintain sternal recumbency, return the animal to its transparent Plexiglas home cag, and keep it on its own with free access to water and food. After the surgery, treat the animal with antibiotics (gentamicin).

11. After the surgery, monitor the animals with daily observation. Check that they walk properly, and that they do not squeak when the experimenter touches the micro-drive array.

4. *In Vivo* Recordings

NOTE: All signals are amplified, sampled at 2 kHz, and band-pass filtered (0.1 - 500 Hz) except for unit activities (sampled at 30 kHz and band-pass filtered (500 - 6 kHz)).

1. Connect the EIB of the integrative micro-drive array to the headstage of a recording device.
2. Advance the tetrodes by turning the screws for a few weeks after surgery. Once the tetrodes are adjacent to the target brain areas, settle the tetrodes into the areas over a period of several days for stable recordings.
3. Monitor the electrical signals while the animal freely moves in a recording chamber.

Representative Results

This method can simultaneously capture bioelectrical signals from multiple organs that represent the neuronal activity of the brain, heart rate, breathing rhythm, and skeletal muscle contractions **(Figure 1)**. **Figure 4** provides representative recording data from a freely moving rat that was freely foraging in a rectangular box (25 × 40 cm^2). The example dataset includes typical behavioral transitions between moving and resting states. A power spectrum was computed from a hippocampal LFP trace by wavelet analysis. The BR signal recorded from the surface area of the olfactory bulb was used to roughly estimate the relative changes in breathing frequencies, such as those that occur during exploratory sniffing behavior.

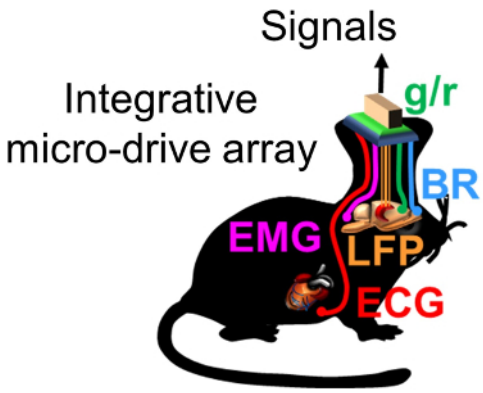

Figure 1: Illustration of the recording system for monitoring multiple brain-body signals from a freely moving rat. All bioelectrical signals (LFP, ECG, EEG, BR signals) from a freely moving rat are collected into the integrative-microdrive array mounted on the head. Please click here to view a larger version of this figure.

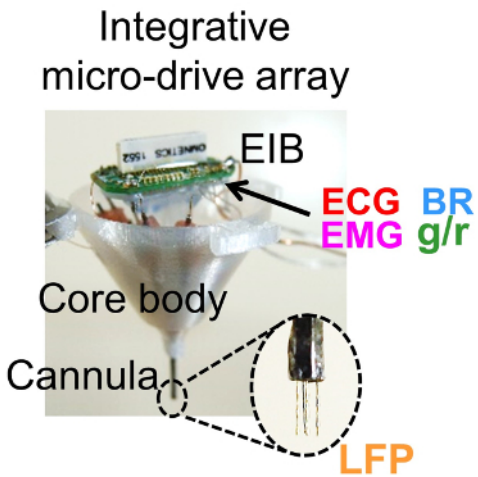

Figure 2: An integrative micro-drive array. All LFP, ECG, EEG, and BR signals are transmitted to the holes on the EIB as indicated by the arrows. The dotted area is magnified in the right panel and displays some of the tetrodes protruding from the micro-drive array that are inserted into the brain tissue. Please click here to view a larger version of this figure.

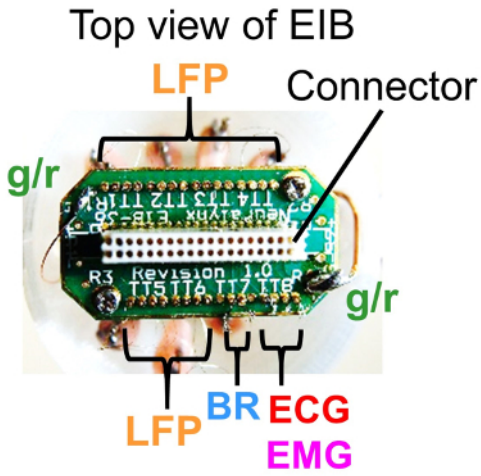

Figure 3: A top view of the EIB. The EIB includes 24 cortical LFP (LFP) channels that are connected to the tetrodes, 2 ECG channels, 2 EMG channels, 2 BR channels, and 2 ground (Gr) channels. All channels except the LFP channels were connected to insulated wires. Please click here to view a larger version of this figure.

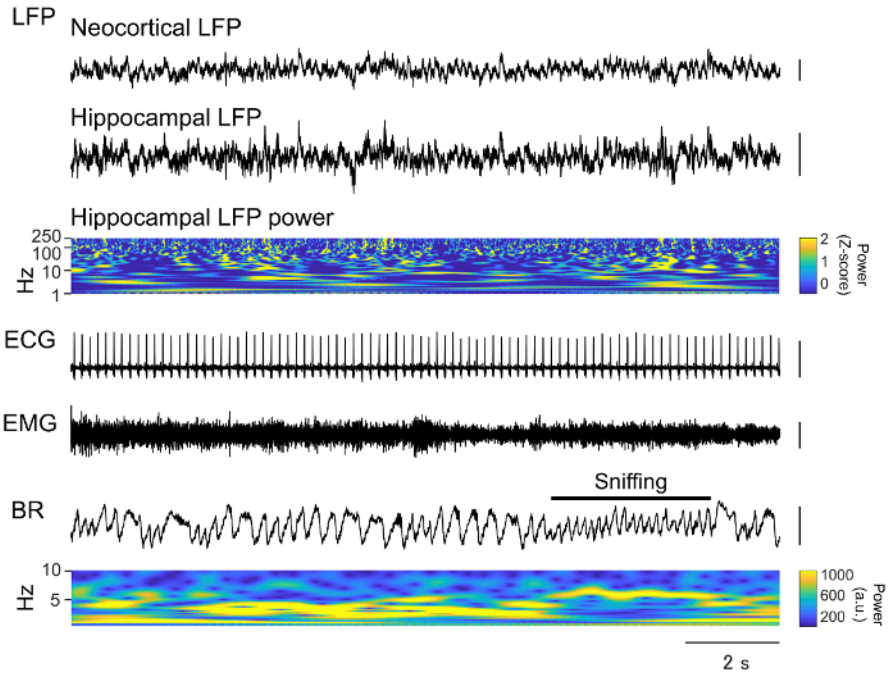

Figure 4: An example of the simultaneous multi-channel recording of bioelectrical signals. (From top to bottom) LFP signals in the somatosensory cortex (scale bar: 250 μV). LFP signals in the hippocampal CA1 region (scale bar: 500 μV). A color-coded power spectrum of the hippocampal LFP trace. An ECG signal (band-pass filtered at 20-200 Hz, scale bar: 500 μV). An EMG signal (band-pass filtered at 100-500 Hz, scale bar: 100 μV). A BR signal (scale bar: 500 μV). A color-coded power spectrum of the BR signal that indicates sniffing behavior as defined by a transient increase in breathing rate. Please click here to view a larger version of this figure.

Discussion

For understanding how the brain modulates peripheral activity levels, and vice versa, large-scale recording methods to simultaneously capture electrical biosignals from multiple body areas are necessary. This study described a surgical procedure, and a recording system for monitoring cerebral local field potentials, heart rates, the magnitude of muscle construction and respiratory rates, which have been improved on a recording system that is used for extracellular recordings in brain tissue. This system collects electrical signals from both the brain and the peripheral organs into a single EIB on an integrative micro-drive array. The preparation of the integrative micro-drive array should be started at least several hours before surgery, as it takes some time. Here, the drive array includes tetrodes for recording brain local field potentials, but the other types of metal electrodes, such as platinum and tungsten electrodes, can be attached to the EIB if the ends of these electrodes are properly soldered. When following the surgical procedures in the protocol, experienced experimenters have been able to complete all the procedures within 2-3 h.

A critical step within this protocol is the positioning of the electrodes on the tissue, especially for the ECG and EMG electrodes. Several training repetitions might be required to obtain stable recordings. To date, all recordings have been stable at least one month after surgery. Experimenters should note that, if the signal-to-noise ratios of the recorded data become low, this problem is frequently due to loose fixations of the ground/reference electrodes, or insufficient soldering of the ends of wires between the EIBs or the other ends of wires. Compared with a conventional electrophysiological recording using some independent devices, the advantages of this method are that (1) it is technically simple to conduct, if trained several times, (2) it does not require a communication system to synchronize multiple devices, (3) it reduces the overall experimental costs, as only one recording device is required, and (4) all recorded data files can be handled by the same processing method and program, which increases the efficiency of data analysis. Furthermore, the methodological concept is applicable to many combinations of multiple brain regions and peripheral organs, including the respiratory system, circulatory system, and autonomic nervous system. It is also applicable to any commercially available electrical recording device. Simultaneous monitoring of systemic physiological activity patterns using this method will be helpful in elucidating neuronal activity patterns in various physiological states against emotional challenges, external sensory modulation, and pathological diseases, leading to an increased understanding of the biological mechanisms underlying the brain-body association.

Disclosures

The authors have nothing to disclose.

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